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ABSTRACTS OF PAPERS AND DISCUSSION

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Glomerular Lesions and the Nephrotic Syndrome in Rabbits given Saccharated Iron Oxide Intravenously, with Special Reference to the Part Played by Intracapillary Precipitates in the Pathogenesis of the Lesions

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Intravascular precipitates, composed at least in part of iron, formed regularly in rabbits given one or more intravenous injections of a saccharated iron oxide preparation, and these lodged in numerous capillaries throughout the body, particularly those of the lungs and kidneys. Large numbers of the brownish precipitates remained in the capillaries of the renal glomeruli during the first few days following injection of the iron, but most of them disappeared after five to seven days, with only moderate amounts of brown pigment remaining in the endothelial cells of the renal glomeruli. Signs of acute injury of the glomerular tufts—namely, pyknosis of some of the endothelial cells, margination of leukocytes within the glomerular capillaries, and slight proliferation of the epithelial cells—also developed some five to seven days following injection of the iron, along with marked proteinuria, which proved transitory if no further injections were given. When the iron preparation was given repeatedly over prolonged intervals, however, the proteinuria persisted and became extreme, and hypoproteinemia developed, often with hypercholesterolemia and transitory edema as well. Histologic studies of the kidneys of rabbits manifesting the nephrotic syndrome, as just described, disclosed that virtually all the renal glomeruli were greatly altered, mainly owing to proliferation of the epithelial cells, together with some fibrosis and atrophy.

Some of the rabbits having marked proteinuria and other functional changes eventually developed azotemia following repeated injections of the iron, and several of them lost weight and died; the renal glomeruli of these animals showed changes like those just described, but the alterations were more extensive.¹

Considered together, the findings provide evidence that intravascular precipitates first occluded the glomerular capillaries for a period of several days following injection of the iron and then largely disappeared from them just prior to the development of morphologic signs of glomerular injury and proteinuria. Hence the possibility was considered that the intracapillary precipitates might have produced acute injury to the walls of the glomerular capillaries through the agency of anoxia. But it is plain that the findings of the present study do not disclose the essential nature of the anatomic change responsible for the proteinuria, or the means whereby this was produced.

The findings as a whole were briefly considered in relation to the pathogenesis of the nephrotic syndrome as it occurs naturally in human beings.

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Nephritis and Nephrosis: Identities and Mechanisms

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Modern classification of disease is based largely on pathogenetic grounds. Accordingly we distinguish between nephritides, nephroses, and other renal diseases.

The identity of the various nephritides is not contested except that More and his associates¹ maintain that the renal lesion caused by foreign protein (serum, globulin) is the same as that of diffuse hemorrhagic glomerular nephritis. This view arose apparently because the basic lesion in this disease may be obscured by thrombosis of loops and its consequences. This complication, however, occurs in many glomerular diseases and hence is not diagnostic. Foreign protein nephritis differs from diffuse hemorrhagic glomerular nephritis in that it is chiefly a disease of the mesangium. Hence, we speak of intercapillary glomerular nephritis.

The nephroses are divided conveniently into tubular and glomerular nephroses as recommended by the speaker many years ago.² The tubular nephroses include storage nephroses characterized morphologically by accumulation in the epithelium of the proximal convolution of urinary components (glycemic, lipemic, hemoglobinemic, cholemic, melanemic nephroses; nephroses due to storage of therapeutic agents such as gelatine), and necrotizing nephroses showing destruction of epithelium of the proximal convolution, or of both the proximal and distal convolution (nephroses due to bacterial toxins, poisons, oxygen deficiency). While the storage nephroses may be clinically silent, the necrotizing nephroses tend to cause renal failure. The identity of the tubular nephroses does not seem to be contested.

The glomerulonephroses include amyloid nephrosis, lipid nephrosis, lupus nephrosis and intercapillary glomerulosclerosis. They are characterized primarily by alteration of glomeruli, and hence by frequent association with the nephrotic syndrome. In typical

cases we find the filtering membrane infiltrated with various protein combinations (amyloid, fibrinoid and others). While no one doubts that amyloid nephrosis and diabetic glomerular sclerosis are nephroses *sui generis*, lipid nephrosis is interpreted by some as a form of glomerular nephritis apparently because the patient with diffuse hemorrhagic glomerular nephritis develops the nephrotic syndrome not infrequently, and because lipid nephrosis terminates in renal failure in at least 50 per cent of the cases. However, all glomerular diseases may cause the nephrotic syndrome, and all may terminate in renal failure. These events can therefore not be cited as criteria of identity.

The etiology of diffuse hemorrhagic glomerular nephritis and lipid nephrosis may well be the same. Diffuse proliferative glomerular nephritis and moderate proteinuria are produced in rats, as shown by the speaker, by small doses of the same anti-kidney serum prepared in rabbits which in large doses causes glomerular degeneration and heavy proteinuria. The pathogenesis of the two diseases, however, is strikingly different. Whether or not one speaks of inflammation, the morbid process in glomerular nephritis is progressive, while that in lipid nephrosis is regressive. But if the pathogenesis is different, and if modern classification of disease is based on pathogenesis, it follows that the two are different diseases.

The various glomerular nephroses, like the glomerular nephritides, may terminate, as has been mentioned, in renal failure. In acute hemorrhagic glomerular nephritis this may be due to obstruction of glomeruli by excessive endothelial proliferation, while in amyloid nephrosis it is caused usually by excessive deposition of amyloid. In most cases, however, renal failure appears to be due to thrombosis of loops with or without exudation into the capsular space. The thrombosis is followed by the formation

within the loops of scars which in lipid nephrosis produce the lobulation of "lobular nephritis." Exudation into the capsular space is followed by crescent formation. If 50 per cent or more of the glomeruli are affected, the urea nitrogen of the blood tends to be 50 mg. per 100 cc. or more. This mechanism is commonly observed in diffuse hemorrhagic glomerular nephritis, intercapillary glomerular nephritis, lipid nephrosis, lupus nephrosis, intercapillary glomerulosclerosis, and even in malignant nephrosclerosis.

DISCUSSION

PAUL KLEMPERER: There is one thing, Dr. Ehrich, which I think has attracted the attention of everyone who has seen lupus in the pre-cortisone era and in the cortisone era. I think the first time cortisone was used in lupus was in 1949. Before that time the occurrence of renal insufficiency with the picture of what in the old terms we would call glomerular nephritis was relatively rare, and the alterations which you describe, which I think we fully agree upon and regard as a form of deposition of something which is protein in the intercapillary space, were the main lesions. Those patients who died with this renal alteration did not show any evidence of renal insufficiency. They showed only blood and albumin in the urine. After cortisone was introduced I would say that practically every patient with lupus dies of renal insufficiency. They live much longer, it is true, and this might be the reason, so I want to ask whether you can correlate this peculiar experience which we have now with your ideas.

WILLIAM E. EHRICH: It appears that renal failure in lupus is not always due to the mechanism which I described. This is an outstanding mechanism in glomerular nephritis, in lipid nephrosis, and in other glomerular diseases. In lupus it occurs also. However, some of our lupus patients who died of renal failure showed extreme wire looping, instead of thrombosis and its consequences. Sometimes all the glomeruli consisted of thick hyaline wire. Observations like these suggest that in these patients wire looping was the reason why they developed

renal failure. It may well be that cortisone permits wire looping to a higher degree than seen previously. We also found erythrocytes in the urine of our patients. The number of erythrocytes varied between 18 and 80 per high power field. The latter finding may be helpful in differential diagnosis of glomerular nephroses.

MAX WACHSTEIN: I would like to ask Dr. Ehrich one technical question pertaining to the use of the combined Hale-PAS stain. I have had considerable difficulty in recognizing the preferential staining of epithelial cells of the glomerulus with colloid iron which has been claimed by Rinehart, Farquhar, Jung and Abul-Haj.³ I had often the impression that endothelial cells as well gave a positive reaction. My second question is probably naive, but could the lipid nephrosis in children be just a form of subacute or chronic nephritis in those children who die from intercurrent infections and do not develop clinical and anatomic renal insufficiency?

WILLIAM E. EHRICH: In regard to the first question, I should like to say that mucopolysaccharide stains, though helpful in the identification of chemical substances, cannot be looked upon as chemically reliable. The blue material of which I spoke was found on the outside of the loops. It was prominent in the experimental animals, but not in the control animals. The lumen of the loops also contained some blue material at times. Rinehart, Farquhar, Jung and Abul-Haj³ observed, I believe, that some of it could be dissolved with hyaluronidase, indicating that it contained acid mucopolysaccharide. Also, we have found that the proximal and distal convolutions of the tubule are readily distinguished in sections stained according to the method of Ritter and Oleson.⁴ While the proximal convolution is lined by a red staining material, the distal convolution shows a blue lining instead. We believe that the latter may contain hyaluronic acid or some other acid mucopolysaccharide, and that this may form the protective colloids contained in urine. This view is supported by the observation that hyaluronidase prevents the precipitation of crys-

talloids (gravel formation) in the urine of persons exposed to stress.

In regard to the second question, it is agreed that patients with subacute glomerulonephritis who die within three months after onset of the disease tend to show many crescents. The more crescents are formed, the more rapidly renal insufficiency ensues. The nephrotic syndrome may be found in 50 per cent of the earlier cases of glomerular nephritis. It seems that about half of the patients takes a hydropic course, while the other half remains anhydropic. It is felt that this point requires further study.

HERBERT CARL STOERK: I should like to ask Dr. Ehrich a question; he showed two slides, one of a DCA-treated and one of a cortisone-treated animal. I should like to know whether they had had previous treatments with horse serum.

WILLIAM E. EHRICH: Yes, one rabbit was treated with horse serum alone. Another was treated with serum and DCA, while a third received serum and cortisone. It was found that DCA aggravated the intercapillary glomerular nephritis caused by horse serum, while cortisone suppressed it.

BEATRICE C. SEEGAL: There is one slide which troubled me and I want to be sure I understand it correctly: when you gave the larger amount of anti-serum and got the nephrotic syndrome early, those animals went on to earlier death, did they not? That has been our experience with the more severe lesions.

WILLIAM E. EHRICH: Rats which received less than 0.1 cc. per gm. of body weight all survived, and many showed proliferative glomerulitis. With doses of 0.1 cc. we had no deaths, but the animals developed

the nephrotic syndrome. With doses higher than 0.1 cc. we had an increasing number of deaths occurring mostly in the first week of the experiment. The experimental period was four weeks.

BEATRICE C. SEEGAL: We are in full agreement that the larger the amount of antibody given, the more certain we are to get the nephrotic syndrome.

WILLIAM E. EHRICH: I should like to comment on the important work of Dr. Seegal and her associates. While Greenspon and Krakower⁵ believed that the antigen was furnished by the basement membrane of the glomeruli, Seegal and Loeb⁶ showed that other tissues which are rich in vessels contain the same antigen. This observation suggests that nephrotic edema is not necessarily due only to leakage of protein through the glomerular filter, but damage of peripheral vessels is involved as well.

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